LINKERS AND CONJUGATES

[0001] The present invention relates to protein and peptide conjugates, and methods of manufacturing the same. More especially, the present invention relates to providing a protein or peptide, linker and an active agent, for example a drug or labelling moiety, to produce a conjugate. Additionally, the present invention provides an improved linker for use in conjugates and methods of introducing said linker into said conjugates. More specifically, the conjugate may be an antibody conjugate, such as an antibody drug conjugate (ADC).

[0002] Protein drug conjugates, in particular antibody drug conjugates, are known to provide targeted delivery of highly potent drugs to specific tissue for treatment. More specifically, ADCs, which typically consist of an antibody linked via a chemical linker to a biologically active cytotoxic or drug payload, are known for use in anticancer treatments. The targeted delivery offered by such protein drug conjugates results from the ability of the antibody or the like to sensitively discriminate between healthy and diseased tissue, thus ensuring safe delivery of the highly potent drug.

[0003] There are currently four ADCs on the market and over 60 others in clinical trials (Beck 2017). However, current approaches for ADC production still have numerous shortcomings and greatly influence an ADCs stability, drug-antibody ratio (DAR) and drug distribution. Nucleophilic bioconjugation at cysteine or lysine residues is pseudorandom, leading to the formation of ADCs that are heterogeneous in terms of the number of cytotoxin molecules incorporated (the DAR) and their locations on the antibody. In addition, the linkage formed via commonly utilized maleimide conjugation to cysteine residues is unstable in circulation leading to premature dissociation of the antibody payload. Such heterogeneous and/or unstable ADCs are associated with unreliable pharmacokinetic profiles and toxic side effects.

[0004] The plasma stability of maleimide-based linkers has been increased by hydrolysis of the succinimide thioether ring through linker modifications or antibody engineering (Lyon 2014). However, an inherently stable linker is preferential. The development of new ADC formats to enable site-selective antibody modification, including the incorporation of engineered cysteine residues (Junutula 2008) and unnatural amino acids (Zimmerman 2014) into the antibody sequence and the use of various enzymatic processes (Chudasama 2016) have produced ADCs with precise DAR and defined attachment points. While effective, these methods are complicated and generally inefficient (Schumacher 2014).

[0005] Recently, disulfide-bridging linkers have emerged for ADC production: a bis-reactive linker moiety undergoes reaction with both thiol residues derived from a reduced cysteine disulfide bond, leading to covalent re-bridging of the protein (Badescu 2014). Such linkers are capable of generating ADCs with more precise DAR and drug distribution as well as reforming covalent bonds between the antibody chains (Schumacher 2014, Behrens 2015, Maruani 2015).

[0006] Of these, dibromomaleimide (DBM) linkers are the most significant; however, cysteine re-bridging reactions with DBM linkers are reversible, thus premature payload release remains a potential issue (Nunes 2015, Chudasma 2011).

[0007] While ADCs are well-known in the art, other protein drug conjugates comprising a protein with the ability to provide targeted delivery of a drug payload are not as well-known. For example, albumin may offer a suitable alternative to antibodies in such protein drug conjugates.

[0008] Albumin consists of three structurally homologous, largely helical domains (I, II and III), each consisting of two subdomains, A and B. Like other mammalian albumins, human albumin contains 17 disulfide bridges and a free thiol at Cys34, which provides the largest fraction of free thiol in blood serum.

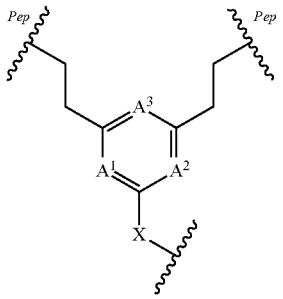
[0009] Additionally, peptide drug conjugates (PDCs) are known and are described, for example, in Wang 2017.

[0010] As well as drug conjugates, the linking of labelling moieties such as fluorophores and biotin tags to proteins and peptides can be useful in techniques such as flow cytometry, Immunofluorescence staining and immunohistochemical staining.

[0011] The present invention aims to provide a disulfide bridging linker platform which addresses the reported stability issues yet retains the advantages of a precise ratio between the active agent and the protein or peptide, and ability to distribute the active agent.

[0012] Accordingly, in a first aspect of the present invention, there is provided a conjugate comprising a protein or a peptide, a linker and an active agent, wherein the linker comprises the moiety of formula (III):

(III)



[0013] wherein two of A^1, A^2 and A^3 are N and the other of A^1, A^2 and A^3 is CH;

[0014] X is selected from N, O and S, and

[0015] Pep indicates where the moiety is linked to the protein or peptide, either directly or indirectly.

[0016] The present invention provides a linker for use in conjugates, with utility in linking proteins or peptides and active agents, for example antibodies and cytotoxins to provide ADC molecules. The linker provides an improved targeted payload of the active agent, and thus may improve the activity of the conjugate where the active agent exerts a biological activity, such as cytotoxicity. Additionally or alternatively, the linker provides the conjugate with increased stability as compared to currently known linker molecules for use in conjugates. This may improve the tolerability of such conjugates.

[0017] The linker may be directly bound to the thio group of a cysteine residue in the peptide or protein, such as an antibody. The linker may re-bridge reduced disulfide bonds in the protein or may be used to staple a peptide.